Effect of spray-dried plasma and lipopolysaccharide exposure on weaned pigs: I. Effects on the immune axis of weaned pigs

K. J. Touchette, J. A. Carroll, G. L. Allee, R. L. Matteri, C. J. Dyer, L. A. Beausang and M. E. Zannelli

J Anim Sci 2002. 80:494-501.

Effect of spray-dried plasma and lipopolysaccharide exposure on weaned pigs: I. Effects on the immune axis of weaned pigs[1,2]

K. J. Touchette*[3], J. A. Carroll†[4], G. L. Allee*, R. L. Matteri†[5], C. J. Dyer†[6], L. A. Beausang‡, and M. E. Zannelli‡

*Department of Animal Sciences, University of Missouri-Columbia 65211; †Animal Physiology Research Unit, Agricultural Research Service, USDA, Columbia, MO 65211; and ‡Pierce-Endogen, Inc., Woburn, MA 01801

ABSTRACT: A study was conducted with 20 weaned barrows (14 d, 4.98 ± .21 kg) to determine the effect of spray-dried plasma (SDP) on the pig's immune response to a lipopolysaccharide (LPS) challenge. After weaning, pigs were fed a diet containing 0 or 7% SDP for 7 d. On d 6 postweaning, all pigs were fitted with a jugular catheter. On d 7 postweaning, the pigs were given an i.p. injection of either saline or LPS (150 μg/kg BW) followed by a 3-h blood collection every 15 min. Following blood collection, all pigs were killed and tissue was collected for mRNA analysis. Additionally, the small intestine was collected for measurement of villus height, crypt depth, and villus height:crypt depth ratio (VCR) at three sites (25, 50, and 75% of the total length). Feeding SDP resulted in reduced ($P < 0.05$) mRNA expression of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) mRNA in the adrenal gland, spleen, hypothalamus, pituitary gland, and liver. Additionally, expression of IL-6 mRNA was reduced ($P < 0.05$) in the spleen and pituitary gland for pigs fed SDP. For pigs fed the diet with SDP, LPS administration did not affect ($P > 0.10$) cytokine mRNA expression, whereas LPS reduced expression of TNF-α mRNA in the spleen and IL-1β mRNA in the adrenal gland, spleen, and thymus for pigs fed the diet without SDP. For pigs fed the diet with SDP, LPS caused serum TNF-α to increase 150-fold compared to a 60-fold increase for pigs fed the diet without SDP. Similarly, interferon-γ (IFN-γ) increased 110-fold for pigs fed the diet with SDP compared to a 16-fold increase for pigs fed the diet without SDP. For pigs fed the diet with SDP, LPS caused major villus atrophy, whereas for pigs fed the diet without SDP, LPS had no effect on intestinal morphology. These results demonstrate that the basal activation of the immune system appears to be less for pigs fed the diet with SDP compared to pigs fed the diet without SDP after weaning. Additionally, for pigs fed the diet with SDP, there appeared to be an overresponse of the immune system following LPS administration, which resulted in major damage to the mucosa of the gastrointestinal tract.

Key Words: Blood Plasma, Immune System, Pigs

 J. Anim. Sci. 2002. 80:494–501

Introduction

Over the past 20 yr, weaning age has been dramatically reduced. As a result of this decreased weaning age, pigs are smaller and have less mature immune and digestive systems at weaning, making them more susceptible to problems in the early postweaning period. Recently, with the addition of spray-dried plasma (**SDP**) to the first diet after weaning, feed intake and growth have improved dramatically (Hansen et al., 1993; Coffey and Cromwell, 1995; de Rodas et al., 1995). The exact mechanism by which SDP improves nursery performance has not been fully elucidated. The fraction that contains immunoglobulins yields performance similar to that from feeding whole SDP (Owen et al.,

[1]The authors express their gratitude to Kurt Holiman, Paul Little, Clyde Morgan, and Jim Ortbals for their technical assistance and to Mona Keaster for her assistance with manuscript preparation.

[2]Mention of a trade name or proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

[3]Current address: Animal Nutritionist, Merrick's Inc., P.O. Box 99, 654 Bridge Street, Union Center, WI 53962.

[4]Correspondence: Room S-107, Animal Science Research Center, Univ. of Missouri, Columbia 65211 (phone: 573-882-6261; fax: 573-884-4798; E-mail CarrollJA@missouri.edu).

[5]Current address: Assistant Area Director, USDA-ARS, Pacific West Area, 800 Buchanan St., Albany, CA 94710-1105.

[6]Current address: Senior Scientist, Monsanto Agricultural Sector, Mail Zone: BB2M, 700 Chesterfield Parkway North, Chesterfield, MO 63198.

Received April 18, 2001.

Accepted September 6, 2001.

1995; Pierce et al., 1995; Weaver et al., 1995). Additionally, research has demonstrated that the response to SDP is greater in a conventional on-site environment than in an off-site environment that is managed to reduce the potential pathogen exposure to weaned pigs (Coffey and Cromwell, 1995; Stahly et al., 1995; Touchette et al., 1996). Thus, it is possible that SDP is able to improve nursery performance by improving the health status of the pig.

Disease is considered to be a major contributing factor associated with poor performance of pigs. Recent reviews by Johnson (1997) and Spurlock (1997) suggest that proinflammatory cytokines released in response to a pathogenic challenge reduce feed intake and growth. Webel et al. (1997) further demonstrated that, following a lipopolysaccharide (**LPS**) challenge, the proinflammatory cytokines tumor necrosis factor-α (**TNF-α**) and interleukin-6 (**IL-6**) were both elevated. This was followed by an increase in plasma urea nitrogen, suggesting that protein degradation occurred. Thus, reducing immunological challenges to growing pigs or improving the pig's ability to cope with an immunological challenge may help reduce protein degradation and improve performance. Thus, the objective of this study was to determine whether feeding SDP would alter the weaned pig's immune response to an LPS challenge.

Materials and Methods

Experimental Design

A total of 20 weaned barrows (Yorkshire × Landrace × Duroc) initially weighing 4.98 ± 0.21 kg and 14 d of age were randomly allotted to one of 10 pens (n = 2 pigs/pen). Each pen was randomly allotted to one of two diets (0 or 7% SDP; n = 5 pens/dietary group). The diets were formulated to contain equal digestible essential amino acids and ME and to meet or exceed NRC (1988) recommendations for other nutrients (Table 1). On d 6 after weaning, all pigs were fitted with a jugular catheter using a nonsurgical procedure (Carroll et al., 1999) and placed back into their respective pens. On d 7 after weaning, all pigs were placed into separate pens for serial blood collection 2 h prior to the challenge period. For the challenge period, pigs were given an i.p. injection of either LPS (150 μg/kg BW) or saline, and then blood samples were collected via the jugular catheter every 15 min for 3 h after the injection. The LPS (*Escherichia coli* serotype 0111:B4; Sigma L-2630, Sigma Chemical, St. Louis, MO) was dissolved in 0.9% (wt/vol) NaCl solution such that 0.3 mL/kg of BW would achieve the desired dosage. After the 3-h blood collection period, all pigs were killed by captive bolt followed by exsanguination for tissue sample collection. Serum was harvested from all blood samples and stored at –80°C until further analyses. All tissue samples to be used for mRNA analysis were collected, immediately placed on Dry Ice, and then stored at –80°C until they were extracted for mRNA analysis.

Table 1. Ingredient composition of diets, % as fed

Item	7% Spray-dried plasma	0% Spray-dried plasma
Ingredient		
Lactose[a]	30.0	30.0
Soybean meal (48% CP)	24.3	24.3
Corn	21.0	21.0
Spray-dried plasma[b]	7.0	—
Soy protein concentrate[c]	—	10.6
Lard	5.0	7.4
Cornstarch	6.0	—
Dicalcium phosphate	2.59	2.75
Blood cells[d]	1.75	1.75
Limestone	0.56	0.42
Salt	0.50	0.50
Mecadox premix[e]	0.25	0.25
Vitamin premix[f]	0.25	0.25
DL-Methionine	0.27	0.24
L-Lysine·HCl	0.15	0.17
Trace mineral premix[g]	0.15	0.15
Copper sulfate	0.09	0.09
L-Threonine	0.14	0.17
Calculated content, %		
Calcium	0.85	0.85
Phosphorus, total	0.80	0.80
CP	21.20	22.50
Lysine[h]	1.39	1.39
Methionine + cystine[h]	0.80	0.80
Threonine[h]	0.91	0.91
Tryptophan[h]	0.27	0.27
ME, Mcal/kg	3.66	3.66

[a]Crystalline source from Land-O-Lakes, Minneapolis, MN.
[b]MP722 from Merrick's, Union Center, WI.
[c]Profine E from Central Soya, Decatur, IL.
[d]Ultracell, from Merrick's, Union Center, WI.
[e]Supplied 55 mg/kg.
[f]Provided the following per kilogram of complete feed: 11,000 IU of vitamin A, 1,100 IU of vitamin D_3, 22 IU of vitamin E, 4 mg of menadione as dimethylpyrimidinol bisulfate, .03 mg of vitamin B_{12}, 28 mg of d-pantothenic acid, and 33 mg of niacin.
[g]Provided the following per kilogram of complete feed: 165 mg of Zn ($ZnSO_4$), 165 mg Fe ($FeSO_4$), 33 mg of Mn ($MnSO_4$), 16.5 mg of Cu ($CuSO_4$), 297 mg of I (CaI_2), and 297 mg Se (Na_2SeO_3).
[h]Calculated true ileal digestibility.

Cytokine Analysis

Serum concentrations of interferon-γ (**IFN-γ**) were determined using a pig IFN-γ ELISA kit as per the instructions of the manufacturer (Pierce-Endogen, Woburn, MA). The dynamic range of the assay was 12.3 to 1,000 pg/mL with a sensitivity of 2.2 pg/mL. The interassay coefficient of variation was less than 8.1% and the intraassay coefficient of variation was less than 8.0%. Serum concentrations of TNF-α were determined using a pig TNF-α ELISA kit as per the instructions of the manufacturer (Pierce-Endogen). The dynamic range of the assay was 38.4 to 1,500 pg/mL with a sensitivity of 6.1 pg/mL. The interassay coefficient of variation was 5.3% and the intraassay coefficient of variation was 3.7%.

Quantification of mRNA

Total RNA was extracted from the hypothalamus, pituitary gland, thymus, spleen, liver, and adrenal

glands (Tri-reagent, Molecular Research Center, Cincinnati, OH) and transferred to a nylon membrane with a slot-blot apparatus (Bio-Dot SF, Bio-Rad Laboratories, Hercules, CA). Hybridization and detection were carried out with a commercially available kit according to the manufacturer's instructions (BrightStar System, Ambion, Austin, TX). Hybridization signal intensities were quantified by densitometry, with target mRNA values expressed relative to 28s ribosomal RNA for each sample. Polymerase chain reaction (PCR) was used to amplify cDNA for interleukin-1 beta (**IL-1β**), IL-6, IL-6 receptor, and TNF-α (RNA-PCR kit, Perkin-Elmer, Foster City, CA). The up- and downstream oligonucleotide primers for PCR amplification were 5′ CAA CGT GCA GTC TAT GGA GT 3′ and 5′ GAG GTG CTG ATG TAC CAG TT 3′ for IL-β (372 bp; GenBank Accession #M86725), 5′ GGA CGC CTG GAA GAA GAT 3′ and 5′ TCT TCA TCC ACT CGT TCT GT 3′ for IL-6 (474 bp; GenBank Accession #M80258), 5′ AGC CCC AGC TCT CCT GCT TC 3′ and 5′ GGC GAC GCA CAT GGA GAC TA 3′ for IL-6 receptor (239 bp; GenBank Accession #AF015116), and 5′ ACC ACG CTC TTC TGC CTA CT 3′ and 5′ AGA TAG TCG GGC AGG TTG AT 3′ for TNF-α (518 bp; GenBank Accession #X57321). The PCR products were subsequently cloned into a T-cloning vector (PCR-II, Inventron, San Diego, CA). The identities of the cDNA clones were confirmed by dideoxy termination sequencing. Biotinylated riboprobes were synthesized from these clones for use in chemiluminescence-based detection (BrightStar System).

Small Intestinal Morphology

The small intestine was dissected free of mesenteric attachments and placed on a smooth surface. Three 5-cm segments at 25, 50, and 75% of the total intestinal length were removed from the intestine. The segments were stored in 10% neutral buffered formalin for 24 h, after which they were cut and histological slides were prepared. Three cross sections (5 μm thick) of each intestinal segment were processed in low-melt paraffin and stained with hematoxylin and eosin. Villous height and crypt depths were quantified using a digitized board coupled to a video monitor receiving output from a video camera mounted on a binocular microscope. Output from the digitizing board was collected with the program Image-Pro Plus (Silver Spring, MD). The villus height was measured from the tip to the base, and then the crypt depth was measured from the base of the villus to the base of the crypt. The villus height:crypt depth ratio (**VCR**) was also calculated. The 10 longest and straightest villi and their associated crypts from each segment were measured. Mean villus heights, crypt depths, and VCR within each segment were calculated for statistical analysis.

Statistical Analysis

Statistical analyses were performed using Statview software (SAS Inst. Inc., Cary, NC) and the theory and rationale of Gardiner and Gettinby (1998). For all mRNA and intestinal morphology data, statistical analysis was performed using analysis of variance and mean comparisons using Fisher's Protected Least Significance Differences. The statistical model for mRNA and intestinal morphology data included the effects of diet (SDP vs no SDP), treatment (saline vs LPS), and their interactions. Serum TNF-α and IFN-γ were analyzed by analysis of variance specific for repeated measures. The statistical model for serum cytokines included the effects of diet (SDP vs no SDP), treatment (saline vs LPS), time, and their interactions. The within-pig error term was used to test for differences among diet, treatment, and diet × treatment. The within-pig-by-time error term was used to test for differences among time, time × diet, time × treatment, and time × diet × treatment. Natural log transformations were conducted on the mRNA data for hypothalamic IL-1β and thymic TNF-α to normalize data prior to further analysis. Data are expressed as the mean ± standard error of the mean.

Results

Pigs fed the diet with SDP (5.57 ± 0.22 kg) and pigs fed the diet without SDP (5.98 ± 0.22 kg) had similar weights on d 7 after weaning. All of the data for cytokine mRNA analysis are shown in Table 2. In the hypothalamus, there tended to be a diet × LPS treatment interaction ($P < 0.09$) for TNF-α mRNA. Pigs fed the diet with SDP had a lower level ($P < 0.001$) of hypothalamic TNF-α mRNA expression regardless of LPS treatment. For pigs fed the diet with no SDP, LPS treatment increased hypothalamic TNF-α mRNA, whereas TNF-α mRNA was decreased for pigs fed the diet with SDP. Hypothalamic IL-1β ($P < 0.05$) mRNA was reduced in pigs fed the diet with SDP compared to pigs fed the diet without SDP. Lipopolysaccharide treatment did not affect IL-1β mRNA in the hypothalamus. Hypothalamic IL-6 mRNA was not affected by either diet or LPS treatment.

In the pituitary gland, expression of TNF-α, IL-6, and IL-1β mRNA were all reduced ($P < 0.05$) for pigs fed the diet with SDP compared to pigs fed the diet without SDP. Lipopolysaccharide treatment did not affect pituitary gland expression of TNF-α, IL-6, or IL-1β mRNA.

In the adrenal gland, there was a diet × LPS treatment interaction ($P = 0.06$) for IL-1β mRNA. For pigs given the saline treatment, those fed the diet with SDP had a lower level of IL-1β mRNA expression than pigs fed the diet without SDP. For pigs given the LPS treatment, IL-1β mRNA expression was reduced in those fed the diet without SDP, but IL-1β mRNA expression was not affected in pigs fed the diet with SDP, resulting in similar levels of IL-1β mRNA. Pigs fed the diet with SDP had a reduced level of adrenal expression of TNF-α mRNA ($P < 0.01$) and tended to have reduced expression of IL-6 mRNA ($P < 0.09$) compared to pigs fed the diet without SDP. Lipopolysaccharide treatment did

Table 2. The effect of spray-dried plasma (SDP) and lipopolysaccharide (LPS) on cytokine mRNA expression (relative amount)

	No LPS		LPS			*P*-values		
Item	No SDP	SDP	No SDP	SDP	SEM	Diet	LPS	Diet × LPS
Hypothalamus								
TNF-α[a]	4.697	3.092	6.214	2.385	0.421	0.0007	0.512	0.088
IL-1β[ab]	5.133	3.047	4.103	2.131	0.183	0.020	0.155	0.987
IL-6[a]	1.883	2.061	2.455	2.313	0.287	0.518	0.978	0.801
Pituitary gland								
TNF-α	3.171	2.221	2.871	2.131	0.183	0.026	0.559	0.753
IL-1β	3.065	2.326	2.669	2.045	0.127	0.005	0.114	0.778
IL-6	4.965	2.106	4.180	2.159	0.551	0.033	0.727	0.690
Adrenal gland								
TNF-α	2.143	0.882	1.650	0.823	0.204	0.009	0.439	0.541
IL-1β	2.211	0.969	1.071	0.868	0.171	0.013	0.028	0.060
IL-6	0.829	0.544	0.813	0.609	0.067	0.090	0.859	0.767
Spleen								
TNF-α	1.906	0.740	1.139	0.836	0.134	<0.0001	0.014	0.003
IL-1β	1.695	0.643	1.012	0.735	0.112	<0.0001	0.101	0.002
IL-6	2.473	1.211	1.751	1.244	0.214	0.040	0.392	0.349
Thymus								
TNF-α[b]	1.004	0.473	0.568	0.497	0.087	0.079	0.209	0.166
IL-1β	2.294	0.879	1.083	1.114	0.177	0.021	0.212	0.018
IL-6	0.753	0.588	0.567	0.602	0.096	0.761	0.686	0.639
Liver								
TNF-α	2.149	0.485	1.140	0.294	0.326	0.034	0.241	0.384
IL-1β	4.288	2.222	3.984	2.798	0.288	0.021	0.849	0.710
IL-6	3.152	1.553	2.126	1.552	0.198	0.002	0.099	0.099

[a]TNF-α = tumor necrosis factor-α, IL-1β = interleukin-1β, and IL-6 = interleukin-6.
[b]Log transformation used to normalize data.

not affect expression of TNF-α or IL-6 mRNA in the adrenal gland.

In the spleen, there was a diet × LPS treatment interaction ($P < 0.01$) for TNF-α and IL-1β mRNA expression. For pigs given the saline treatment, those fed the diet with SDP had reduced expression of TNF-α and IL-1β mRNA compared to pigs fed the diet without SDP. Lipopolysaccharide treatment reduced both TNF-α and IL-1β mRNA expression in pigs fed the diet without SDP but did not affect these mRNA levels for pigs fed the diet with SDP, resulting in similar expression levels of these mRNA. Pigs fed the diet with SDP had reduced IL-6 mRNA expression ($P < 0.05$) in the spleen compared to pigs fed the diet without SDP. Lipopolysaccharide treatment did not affect IL-6 mRNA expression levels in the spleen.

In the thymus, there was a diet × LPS treatment interaction for IL-1β mRNA expression ($P < 0.05$). For pigs given the saline treatment, those fed the diet with SDP had lower IL-1β mRNA expression than pigs fed the diet without SDP. Lipopolysaccharide treatment reduced expression of IL-1β mRNA for pigs fed the diet without SDP but did not affect IL-1β mRNA expression levels in pigs fed the diet with SDP, resulting in similar levels of this mRNA. Thymic TNF-α mRNA expression was not affected by LPS treatment but tended ($P < 0.08$) to be lower in pigs fed the diet with SDP. Neither diet nor LPS treatment affected IL-6 mRNA expression in the thymus.

In the liver, there tended to be a diet × LPS treatment interaction for IL-6 mRNA expression ($P < 0.10$). For pigs given the saline treatment, those fed the diet with SDP had lower IL-6 mRNA expression levels than pigs fed the diet without SDP. Lipopolysaccharide treatment reduced IL-6 mRNA expression in pigs fed the diet without SDP but did not affect IL-6 mRNA expression levels in pigs fed the diet with SDP, resulting in similar levels of this mRNA. Feeding a diet with SDP reduced ($P < 0.05$) expression of liver TNF-α and IL-1β mRNA compared to a diet without SDP, whereas LPS treatment did not affect expression of these mRNA levels in the liver.

There was a diet × LPS treatment interaction for both serum TNF-α (Figure 1) and IFN-γ (Figure 2). The serum TNF-α response to LPS was much greater in pigs fed the diet with SDP (peak was 15.7 ng/mL at 1.25 h after the challenge) than in pigs fed the diet without SDP (peak was 6.3 ng/mL at 1.5 h after the challenge). Similarly, IFN-γ was greater in pigs fed the diet with SDP (1.75 ng/mL) than in pigs fed the diet without SDP (0.24 ng/mL) 3 h after LPS administration. For pigs given the saline treatment, diet did not affect either serum TNF-α or IFN-γ.

Feeding SDP did not affect villus height but reduced crypt depth in pigs given the saline treatment (Table

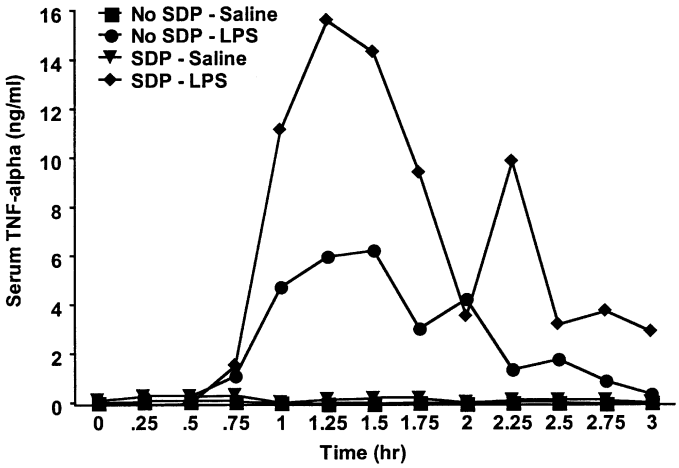

Figure 1. The effect of spray-dried plasma (SDP) and lipopolysaccharide (LPS) treatment on serum tumor necrosis factor-α (TNF-α). There was a significant diet × LPS treatment interaction ($P < 0.05$), such that pigs fed the diet with SDP responded more than pigs fed the diet without SDP. Error bars are omitted for presentation purposes. Pooled SEM was 0.283 ng/mL.

3). This resulted in an improved VCR for pigs fed the diet with SDP. For pigs fed the diet without SDP, LPS did not affect villus height, crypt depth, or VCR. For pigs fed the diet with SDP, LPS caused severe intestinal mucosa damage (Figure 3), resulting in a decreased villus height and VCR, with no effect ($P > 0.10$) on crypt depth. Additionally, four of the five pigs fed the diet with SDP and given the LPS treatment exhibited signs of gastric infarction, whereas none of the other pigs exhibited this condition.

Discussion

Feeding SDP altered the pigs' response to an immunological challenge. For pigs fed the diet with SDP, LPS caused serum TNF-α to increase 150-fold, compared to a 60-fold increase for pigs fed the diet without SDP. Similarly, IFN-γ increased 110-fold for pigs fed the diet with SDP, compared to a 16-fold increase for pigs fed the diet without SDP. There were no basal effects of diet on these serum cytokines. Indeed, for pigs fed the diet with SDP, there appears to have been an overresponse in production of these cytokines that may be associated with the gastric infarction and severe intestinal damage.

Lipopolysaccharide treatment caused gastric infarctions in four of the five pigs fed the diet with SDP. For pigs fed the diet with no SDP, there were no indications of gastric infarctions following LPS administration. Gastric infarction has been previously associated with a bacterial infection (Barker et al, 1993; Cook, 1996). Additionally, LPS caused villus atrophy for pigs that were fed the diet with SDP, but there was no change in villus height for pigs fed the diet without SDP. This atrophy of the villi was likely a result of increased serum TNF-α. Previous research has reported that if a mouse is given anti-TNF-α antibodies mucosal destruction is eliminated following an LPS challenge (Mathison et al., 1988; Remick et al., 1990; DeForge et al., 1994), suggesting that TNF-α plays an important role in mediating mucosal destruction associated with an immunological challenge. In this study, it appears that the over-production of TNF-α may have resulted in the tissue damage seen for pigs fed the diet with SDP. For pigs fed the diet with no SDP, TNF-α increased dramatically, but not to an extent to cause mucosal damage in the gastrointestinal tract.

Tissue expression of cytokine mRNA of the pigs given the saline treatments may be a good indicator of immune status after the 1st wk following weaning. Tumor necrosis factor-α and IL-1β mRNA in all tissues and IL-6 mRNA in the adrenal gland, spleen, pituitary gland, and liver were all reduced in pigs fed the diet with SDP compared to pigs fed the diet without SDP. This may suggest that activation of the immune system is reduced when SDP is included in the diet of the weaned pig.

The differences in basal immune system activation may be an indicator of the differences seen in response to LPS administration. DeForge et al. (1994) created two groups of mice differing in immune status. The first group was "naive," whereas the second group was "primed" with Complete Freund's Adjuvant. They reported that "naive" mice had a much greater TNF-α and IL-6 release than "primed" mice following LPS administration. They also noted that "naive" mice sustained much more mucosal damage in the lungs and small intestine than the "primed" mice. Additionally, Hadid et al. (1995) evaluated the effect of repeated

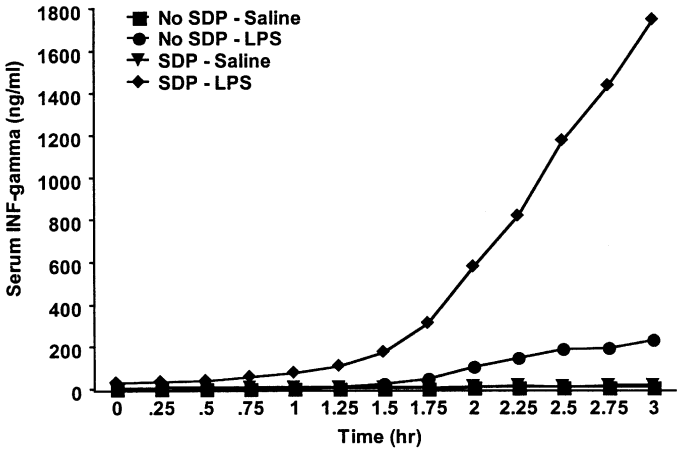

Figure 2. The effect of spray-dried plasma (SDP) and lipopolysaccharide (LPS) treatment on serum interferon-γ (IFN-γ). There was a significant diet × LPS treatment interaction ($P < 0.05$), such that pigs fed the diet with SDP responded more than pigs fed the diet without SDP. Error bars are omitted for presentation purposes. Pooled SEM was 29 ng/mL.

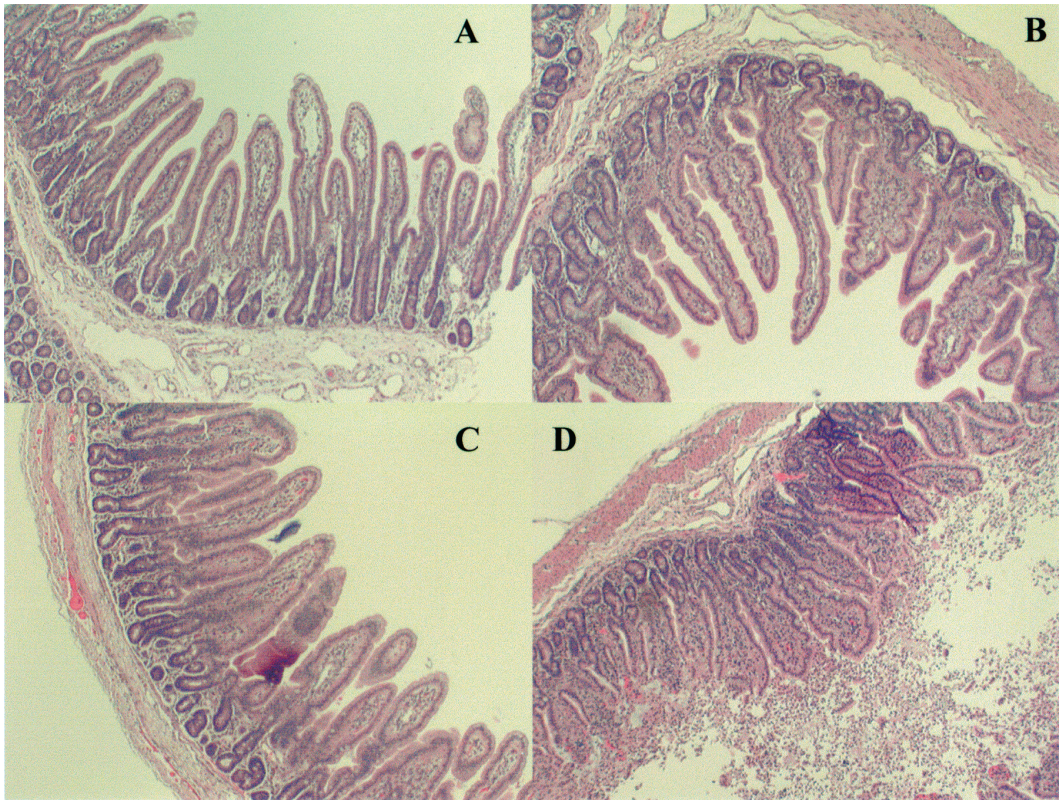

Figure 3. Histological slides of the mucosal epithelium of the small intestine. (A) Pig fed the diet with no spray-dried plasma (SDP) and given the saline injection; (B) pig fed the diet with 7% SDP and given the saline injection; (C) pig fed the diet with no SDP and given the lipopolysaccharide (LPS) injection; and (D) pig fed the diet with 7% SDP and given the LPS injection.

daily LPS challenges in mice. They found that, after the third dose of LPS, the release of TNF-α was reduced compared to the first dose of LPS. Similarly, Erroi et al. (1993) evaluated cytokine production in mice pretreated with LPS for four consecutive days. On d 6, LPS administration to the pretreated mice reduced serum IL-6, TNF-α, IFN-γ, IL-1α, and IL-1β compared to mice that had not been pretreated. Tumor necrosis factor-α production was completely eliminated, whereas IFN-γ was reduced by 85%. These studies provide clear evidence that an animal that has been exposed to prior immune challenges will have a reduced response to a future immunological challenge. Thus, pigs fed SDP may have been similar to the "naive" mice, whereas pigs fed the diet without SDP may have been similar to the "primed" or repeatedly LPS-challenged mice.

The exact mechanism by which this immunological response is reduced is unclear. It is possible that IL-4 (Lee et al., 1990; Curfs et al., 1997), IL-10 (Randow et al., 1995; Walley et al., 1996; Curfs et al., 1997), or transforming growth factor-β (Palladino et al, 1990; Randow et al., 1995; Curfs et al., 1997) may have been up-regulated in the pigs fed the diet with no SDP. These anti-inflammatory cytokines have been implicated in causing LPS tolerance similar to that seen in this study. Thus, a "naive" group of animals may be lacking the negative feedback system readiness compared to an immune-stimulated group of animals, making this group more susceptible to overproduction of inflamma-

Table 3. Effect of spray-dried plasma (SDP) and lipopolysaccharide (LPS) on intestinal morphology[a]

	0% SDP		7% SDP			*P*-value		
Item	No LPS	LPS	No LPS	LPS	SEM	Plasma (P)	LPS	P × LPS
Villus height	197.38	193.42	188.05	166.78	7.70	0.02	0.11	0.27
Crypt depth	84.75	87.40	64.49	70.63	2.67	0.001	0.11	0.52
Villus:crypt ratio	2.46	2.30	3.00	2.44	0.12	0.008	0.005	0.11

[a]Values are means of five pigs/treatment.

tory cytokines in response to a major pathogen challenge.

For pigs fed the diet with SDP, LPS did not affect tissue expression of cytokine mRNA. For pigs fed the diet without SDP, LPS reduced expression of IL-1β mRNA in the adrenal gland, spleen, and thymus and reduced expression of TNF-α mRNA in the spleen. These results suggest that there has been negative feedback regulation, because cytokines were increased under both conditions. Previous results demonstrate that immediately after LPS administration tissue expression of cytokine mRNA are increased and then decrease later (Frankenberger et al., 1995). In this study, serum levels of TNF-α returned to near baseline values by the end of the 3-h sampling period. Thus, the tissue expression of cytokine mRNA had probably already undergone negative feedback. For pigs fed the diet without SDP, this negative feedback mechanism may have been stronger, as suggested by lower expression of cytokine mRNA in pigs given LPS compared to pigs given the saline injection. Therefore, the differences in mRNA profiles observed between the present study and those previously reported may be associated with the time of tissue collection after the LPS challenge.

There are two potential mechanisms by which SDP may prevent stimulation of the immune system in the 1st wk following weaning. The first may be a direct effect of SDP in preventing antigen growth or colonization in the small intestine. Previous research has demonstrated that the fraction of SDP that contains active immunoglobulins and a diet containing whole SDP yield similar growth performance in pigs (Owen et al., 1995; Pierce et al., 1995; Weaver et al., 1995). Possibly, the immunoglobulins that are in SDP may bind to potential antigens in the lumen of the small intestine and prevent their attachment or growth. This may reduce insults on the pig's immune system, thus reducing immune activation and improving feed intake. The second may be an indirect effect of SDP helping the pig's mucosal integrity. Previous research has indicated that SDP promotes intestinal growth, as indicated by an improvement in villus height and VCR (Spencer et al., 1997; Touchette et al., 1997). An important function of the small intestine is to provide a physical barrier that limits the migration of pathogenic organisms outside the intestinal lumen. Thus, feeding SDP may improve this barrier function and prevent potential antigens from entering the pig and stimulating the immune system.

These two mechanisms may work to reduce the stimulation of the pig's immune system during the 1st wk after weaning. It has been demonstrated that immune system activation is associated with reduced feed intake and growth (Williams et al., 1993; Coffey and Cromwell, 1995). Thus, reducing activation of the immune system by providing SDP in the diet of weaned pigs may improve feed intake and growth.

It should be noted, however, that in the present study we did not observe a difference in the growth of the SDP-supplemented pigs compared to those pigs fed a diet without SDP. We speculate that, although the bacterial load within the environment in which the pigs were maintained was sufficient to alter immune function, it was not significant enough to reduce growth. Although previous studies have reported that the growth response to SDP is greater in a conventional on-site environment than in an off-site environment that is managed to reduce pathogen exposure (Coffey and Cromwell, 1995; Stahly et al., 1995; Touchette et al., 1996), no studies to our knowledge have investigated the potential immunological effect of SDP supplementation in these two diverse environments. Therefore, future studies are needed that specifically investigate the effect of SDP on immune function, feed intake, and growth of pigs that are maintained in pathogen-free environments and in environments in which the pathogenic load has been quantified.

If feeding SDP in the 1st wk after weaning reduces activation of the pig's immune system, it appears to make the pig more susceptible to a future major immunological challenge, possibly because the negative feedback system is not yet in place or is slow in responding. Given that the mode of injection in this study was i.p. and that the level of challenge was extremely high, it is difficult to determine how a pig fed a diet with SDP would react in a typical production environment. This environment would contain lower amounts of pathogen exposure and the mode of entry into the pig's system would most likely be via nasal passage or the gastrointestinal system.

Implications

This study demonstrates that basal immune parameters for pigs fed a diet with spray-dried plasma are depressed compared to those of pigs fed a diet without spray-dried plasma. Additionally, this study indicates that the greater response in lipopolysaccharide-induced inflammatory cytokines observed in pigs fed spray-dried plasma resulted in major damage to the mucosa of the gastrointestinal tract. This suggests that pigs fed spray-dried plasma may be more susceptible to major immunological challenges. However, reduction of the immune system activation may be the mechanism by which feed intake and growth rate are improved under commercial conditions when spray-dried plasma is included in the postweaning diet.

Literature Cited

Barker, I. K., A. A. Van Dreumel, and N. Palmer. 1993. The alimentary system. In: K. V. F. Jubb, P. C. Kennedy, and N. Palmer (ed). Pathology of Domestic Animals. vol. 2. 4th ed. pp 1–318. Academic Press, New York.

Carroll, J. A., J. A. Daniel, D. H. Keisler, and R. L. Matteri. 1999. Non-surgical catheterization of the jugular vein in young pigs. Lab. Anim. 33:129–134.

Coffey, R. D., and G. L. Cromwell. 1995. The impact of environment and antimicrobial agents on the growth response of early-weaned pigs to spray-dried porcine plasma. J. Anim. Sci. 73:2532–2539.

Cook, R. W. 1996. Oesophogus and stomach. In: L. D. Sims and R. W. Glastonbury (ed). Pathology of the Pig: A Diagnostic Guide. Pig Research and Development Corp., Barton, Australia.

Curfs, J. H. A. J., J. F. G. M. Meis, and J. A. A. Hoofkamp-Korstanje. 1997. A primer on cytokines: sources, receptors, effects, and inducers. Clin. Microbiol. Rev. 10:742–780.

DeForge, L. E., E. Takeuchi, D. T. Nguyen, and D. G. Remick. 1994. Immunological priming attenuates the *in vivo* pathophysiological response to lipopolysaccharide. Am. J. Pathol. 144:599–611.

de Rodas, B. Z., K. S. Sohn, C. V. Maxwell, and L. J. Spicer. 1995. Plasma protein for pigs weaned at 19 to 24 days of age: Effect on performance and plasma insulin-like growth factor I, growth hormone, insulin, and glucose concentrations. J. Anim. Sci. 73:3657–3665.

Erroi, A., G. Fantuzzi, M. Mengozzi, M. Sironi, S. F. Orencole, B. D. Clark, C. A. Dinarello, A. Isetta, P. Gnocchi, M. Giovarelli, and P. Ghezzi. 1993. Differential regulation of cytokine production in lipopolysaccharide tolerance in mice. Infect. Immun. 61:4356–4359.

Frankenberger, M., H. Pechumer, and H. W. L. Ziegler-Heitbrock. 1995. Interleukin-10 is upregulated in LPS tolerance. J. Inflamm. 45:56–63.

Gardiner, W. P., and G. Gettinby. 1998. Experimental Design Techniques in Statistical Practice. Horwood Publishing, Chichester, U.K.

Hadid, R., E. Spinedi, T. Daneva, G. Grau, and R. C. Gaillard. 1995. Repeated endotoxin treatment decreases immune and hypothalamo-pituitary-adrenal axis responses: Effect of orchidectomy and testosterone therapy. Neuroendocrinology 62:348–355.

Hansen, J. A., J. L. Nelssen, R. D. Goodband, and T. L. Weeden. 1993. Evaluation of animal protein supplements in diets of early-weaned pigs. J. Anim. Sci. 71:1853–1862.

Johnson, R. W. 1997. Inhibition of growth by pro-inflammatory cytokines: An integrated view. J. Anim. Sci. 75:1244–1255.

Lee, J. D., S. G. Swisher, E. H. Minehart, W. H. McBride, and J. S. Economou. 1990. Interleukin-4 downregulates interleukin-6 production in human peripheral blood mononuclear cells. J. Leukoc. Biol . 47:457–479.

Mathison, J. C., E. Wolfson, and R. J. Ulevitch. 1988. Participation of tumor necrosis factor in the mediation of gram negative bacterial lipopolysaccharide-induced injury in rabbits. J. Clin. Investig. 81:1925–1937.

NRC. 1988. Nutrient Requirements of Swine. 9th ed. National Academy Press, Washington, DC.

Owen, K. Q., J. L. Nelssen, R. D. Goodband, M. D. Tokach, K. G. Friesen, B. T. Richert, J. W. Smith, and L. E. Russell. 1995. Effects of various fractions of spray-dried porcine plasma on performance of early-weaned pigs. J. Anim. Sci. 73(Suppl. 1):81 (Abstr.).

Palladino, M. A., R. E. Morris, H. F. Starnes, and A. D. Levinson. 1990. The transforming growth factor-betas. A new family of immunoregulatory molecules. Ann. N. Y. Acad. Sci. 593:181–187.

Pierce, J. L., G. L. Cromwell, M. D. Lindemann, and R. D. Coffey. 1995. Assessment of three fractions of spray-dried plasma on performance of early-weaned pigs. J. Anim. Sci. 73(Suppl. 1):81 (Abstr.).

Randow, R., U. Syrbe, C. Meisel, D. Krausch, H. Zuckerman, C. Platzer, and H. Volk. 1995. Mechanism of endotoxin desensitization: Involvement of interleukin-10 and transforming growth factor β. J. Exp. Med. 181:1878–1882.

Remick, D. G., R. M. Streiter, M. K. Eskandari, D. T. Nguyen, M. A. Genord, C. L. Raiford, and S. L. Kunkel. 1990. Role of tumor necrosis factor-alpha in lipopolysaccharide-induced alterations. Am. J. Pathol. 136:49–60.

Spencer, J. D., K. J. Touchette, H. Liu, G. L. Allee, M. D. Newcomb, M. S. Kerley, and L. W. Pace. 1997. Effect of spray-dried plasma and fructooligosaccharide on nursery performance and small intestinal morphology of weaned pigs. J. Anim. Sci. 75(Suppl. 1):199 (Abstr.).

Spurlock, M. E. 1997. Regulation of metabolism and growth during immune challenge: An overview of cytokine function. J. Anim. Sci. 75:1773–1783.

Stahly, T. S., D. R. Cook, S. G. Swenson, N. H. Williams, and D. R. Zimmerman. 1995. Growth response of pigs to dietary plasma protein (PP) additions as influenced by pig antigen exposure and PP source. J. Anim. Sci. 73(Suppl. 1):81 (Abstr.).

Touchette, K. J., G. L. Allee, and M. D. Newcomb. 1996. The effects of plasma, lactose, and soy protein source fed in the phase I diet on nursery performance. J. Anim. Sci. 74(Suppl. 1):170 (Abstr.).

Touchette, K. J., G. L. Allee, M. D. Newcomb, L. W. Pace, and M. R. Ellersieck. 1997. Impact of feed intake and spray-dried plasma on nursery performance and intestinal morphology of weaned pigs. J. Anim. Sci. 75(Suppl. 1):198 (Abstr.).

Walley, K. R., N. W. Lukacs, T. J. Standiford, R. M. Streiter, and S. L. Kunkel. 1996. Balance of inflammatory cytokines related to severity and mortality of murine sepsis. Infect. Immun. 64:4733–4738.

Weaver, E. M., L. E. Russell, and M. D. Drew. 1995. The effect of spray-dried plasma fractions on performance of newly weaned pigs. J. Anim. Sci. 73(Suppl. 1):81 (Abstr.).

Webel, D. M., B. N. Finck, D. H. Baker, and R. W. Johnson. 1997. Time course of increased plasma cytokines, cortisol, and urea nitrogen in pigs following intraperitoneal injection of lipopolysaccharide. J. Anim. Sci. 75:1514–1520.

Williams, N. H., T. S. Stahly, and D. R. Zimmerman. 1993. Impact of immune activation and dietary amino acid regimen on nitrogen retention in pigs. J. Anim. Sci. 71(Suppl. 1):171 (Abstr.).